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Molecular Docking of Phytochemicals from *Adhatoda vasica* Against Caries, Periodontitis and Inflammatory Mediators – A Computational Study

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Abstract

Orthodontic treatment is associated with pain, difficulty in plaque control, caries incidence, and slow movement of teeth. Authors hypothesize that the answer to these questions may lie in phytochemicals which are a huge unexplored area. Due to previous reports on Adhathoda vasica about its anti-inflammatory and antimicrobial properties, its use in orthodontics is explored in the dry lab scenario. This can be considered as a preliminary step to study the herb in great detail for use in orthodontic therapy. Computational algorithms from Auto Dock version 4 were used in the study. Vasicine, Vasinone, Vasicoline and Anisotine were analysed against Glucosyltransferase (GFT) of Streptococcus mutans, Gingipain K - Porphyromonas gingivalis, FIM A of Porphyromonas gingivalis, TNF ALPHA and Prostaglandin H synthases. Compounds exerted promising inhibiting action against Streptococcus mutans, Gingipain K, FimA, TNF-alpha and prostaglandin E2. The said phytochemicals - Vasicine, Vasicinone, Vasicoline and Anisotine can further be explored for proper delivery to saliva, periodontal region and to bone for effective use during and after orthodontic treatment.

Keywords*: Adhatoda vasica, docking, Glucosyltransferase, Gingipain K - Porphyromonas gingivalis, FIM A of Porphyromonas gingivalis, TNF ALPHA and Prostaglandin H synthases.*

Introduction

In nature, caries and periodontitis do not commonly exist together, except in cases of very poor oral hygiene [1]. This is because distinct flora are associated with caries and periodontitis which antagonize each other [2] However, rare situations like orthodontic treatment cause an increase in both cariogenic and periodontal flora. As mentioned, orthodontic treatment, especially using fixed appliances greatly hinders the maintenance of oral hygiene. Therefore, various aids are used to promote this cleaning process to prevent caries and periodontitis, which are equally deleterious to the patient [3]

agent that can achieve all this. For controlling pain, NSAIDs are used and duration may not

Of various modalities used to treat the situation, after physical techniques of brushing, chemical methods come into play. Mouthwashes play a major role in plaque control in these situations. Though they are successful, numerous adverse events like damaging mucosa, staining of teeth, and altering taste perception are associated with it [4].

In another direction, many patients refuse orthodontic treatment due to the pain associated with it and its long duration [5]. If these issues can be addressed, acceptance of orthodontic treatment would increase and more patients would benefit from it. In the present scenario, there is no single be effectively controlled by drugs or chemicals [6].

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Authors hypothesize that the answer to these questions may lie in phytochemicals which are a huge unexplored area. In that direction, due to previous reports on *Adhathoda vasica* about its anti-inflammatory and antimicrobial properties, its use in orthodontics is explored in the dry lab scenario. This can be considered as a preliminary step to study the herb in great detail for use in orthodontic therapy.

Materials and Methods

Protein-Ligand Docking

Computational algorithms predicting the possibility of leads over selected targets were performed by using a standard docking tool (Auto Dock version 4). The outcome of screening was ascertained by observing the binding affinity and interaction pattern of the lead with targets [7].

Protein Preparation

The three-dimensional structure of the target proteins illustrated in Figure 1 was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB). The retrieved protein construct was subjected to surface optimization by removing the preloaded lead candidate and subsequent water molecules cleaved. Gasteiger charges were computed with additional polar hydrogen atoms, and the merging of non-polar and rotatable bonds was defined using AutoDock 4 [8].

Ligand Model Preparation

Two- and three-dimensional structures of selected ligands viz. Vasicine, Vasicinone, Vasicoline and Anisotine subjected to molecular docking investigation were constructed by using ChemDraw sketch software. Figure 2 represents the 2D and 3D structures of selected ligand molecules subjected to molecular docking investigation.

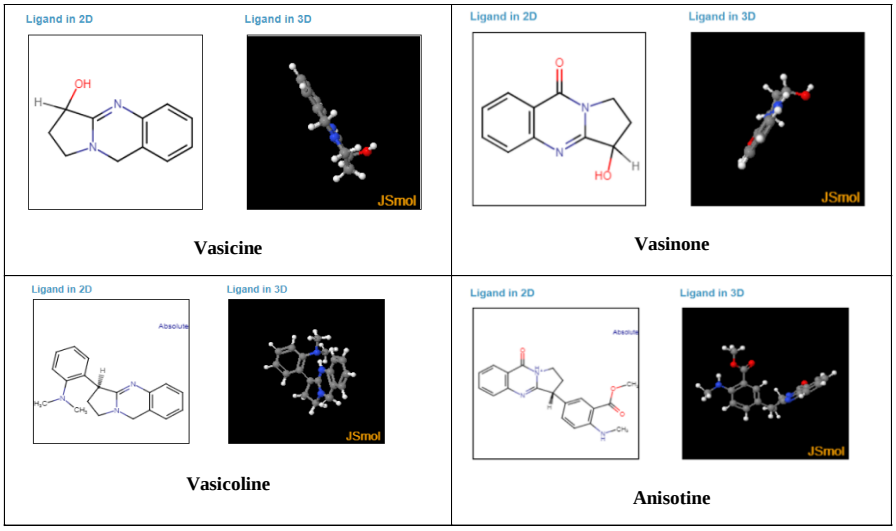

Fig. 1. 2D and 3D Structure of Phytocomponents

Table 1. Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar Weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable Bonds
Vasicine	188.23 g/mol	$C_{11}H_{12}N_2O$	1	2	0
Vasicinone	202.213 g/mol	$C_{11}H_{10}N_2O_2$	1	3	0
Vasicoline	291.4 g/mol	$C_{19}H_{21}N_3$	0	2	2
Anisotine	349.4 g/mol	$C_{20}H_{19}N_3O_3$	1	5	4

Docking Simulations

In-silico docking predictions were performed using a licensed version of AutoDock 4. The virtual screening tool works behind the algorithm to predict the possible interaction between the functional group of the lead with the active site (amino acid residue) of the protein target. The three-dimensional conformation of target proteins retrieved from RCSB was subjected to computational docking by using AutoDock 4. Molecular energy and free dynamics calculation were optimized using the auto dock algorithm and protein charge calculation set with the Gasteiger simulation module. Solvation parameters and other respective polar hydrogens were added to the receptor for the preparation of protein for successive docking simulation. Since ligands are phytotherapeutics and not of peptide origin hence, Gasteiger charges were essentially applied and then non-polar hydrogens were merged. As per the requirement of the AutoDock programming, the pre-calculated grid maps were set for one for each atom type, present in the ligand being docked as it stores the potential energy arising. The docking pocket was set with affinity (grid) maps of 70×70×70 Å grid points and with 0.375 Å. Each docking calculation was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [9, 10].

Table 2. Details of Targets

S. No.	Purpose	PDB	Name of Target
1	Anti-Microbial activity - Caries	3AIC [11]	Glucosyltransferase (GFT) of *Streptococcus mutans*
2	Anti-microbial activity - Periodontal	6I9A	Gingipain K - *Porphyromonas gingivalis*
3	Anti-microbial activity - Periodontal	4Q98	FIM A of *Porphyromonas gingivalis*
4	Analgesic / Anti-Inflammatory Activity	2AZ5	TNF ALPHA
5	Anti-Inflammatory Activity	1IGX	Prostaglandin H synthases

Receptor Structures

The crystalline structure of the target proteins was retrieved from the protein data bank and protein clean-up process was done and essential missing hydrogen atoms were added. Different orientation of the lead molecules concerning the target protein was evaluated by the Autodock program and the best dock pose was selected based on the interaction study analysis.

Docking calculations were carried out for retrieved phytocomponents against target protein 3CL pro. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools.[10] Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [12]. The initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set at 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [13, 14, 15].

In-silico docking simulations were performed by using Auto Dock version 4. The molecular interaction of residual amino acids with the core functional groups determines the efficacy of the lead molecules. Three dimensional pharmacophores of FDA-approved lead molecules were subjected to virtual screening against selected protein target Gingipain K - *Porphyromonas gingivalis* with PDB 6I9A retrieved from RCSB by using Auto Dock 4. Docking grids were set with the pocket size measuring maps of 70×70×70 Å grid points and with 0.375 Å. Each docking calculation is set to run with 10 different cycles after a maximum of 250000 energy evaluations. The population size was set at 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [7, 16].

Results

Table 3. Summary of the Molecular Docking Studies of Compounds Against Glucosyltransferase (GFT) of *Streptococcus mutans*- PDB- 3AIC

Compounds	Binding Free Energy Kcal/mol	Inhibition Constant Ki µM (*mM)(**nM)	Electrostatic Energy Kcal/mol	Intermolecular Energy Kcal/mol	Total Interaction Surface
Vasicine	-7.02 kcal/mol	7.20 uM	-2.79 kcal/mol	-7.31 kcal/mol	539.729
Vasicinone	-5.01 kcal/mol	212.78 uM	-0.07 kcal/mol	-5.31 kcal/mol	464.886
Vasicoline	-9.24 kcal/mol	169.65 nM	-2.70 kcal/mol	-9.42 kcal/mol	671.964
Anisotine	-7.04 kcal/mol	6.94 uM	-0.01 kcal/mol	-7.55 kcal/mol	662.994

Table 4. Amino acid Residue Interaction of Lead against Glucosyltransferase (GFT) of *Streptococcus mutans*- PDB- 3AIC

Compounds	Interaction	Amino Acid Residues										
Vasicine	3	433 LEU	475 ARG	477 ASP	478 ALA	481 ASN	515 TRP	517 TRP	587 HIS	588 ASP	916 TYR	
Vasicinone	3	433 LEU	477 ASP	478 ALA	480 ASP	481 ASN	515 TRP	517 TRP	588 ASP			
Vasicoline	3	430 TYR	433 LEU	475 ARG	477 ASP	480 ASP	481 ASN	515 TRP	517 TRP	587 HIS	588 ASP	916 TYR
Anisotine	3	433 LEU	477 ASP	515 TRP	517 TRP	588 ASP	589 SER	592 GLN	593 ASP	610 TYR	907 PHE	916 TYR

Table 5. Summary of the Molecular Docking Studies of Compounds Against Gingipain K - *Porphyromonas gingivalis* – PDB 6I9A

Compounds	Binding Free Energy Kcal/mol	Inhibition Constant Ki µM (*mM)(**nM)	Electrostatic Energy Kcal/mol	Intermolecular Energy Kcal/mol	Total Interaction Surface
Vasicine	-5.94 kcal/mol	44.19 uM	-0.80 kcal/mol	-6.24 kcal/mol	447.239
Vasicinone	-5.93 kcal/mol	45.06 uM	-0.10 kcal/mol	-6.23 kcal/mol	424.733
Vasicoline	-7.49 kcal/mol	3.26 uM	-0.58 kcal/mol	-7.23 kcal/mol	577.76
Anisotine	-5.65 kcal/mol	72.15 uM	-0.03 kcal/mol	-6.32 kcal/mol	592.433

Table 6. Amino Acid Residue Interaction of Leads Against Gingipain K - *Porphyromonas gingivalis* – PDB 6I9A

Compounds	Interaction	Amino acid Residues						
Vasicine	2	442 THR	444 HIS	477 CYS	511 SER	512 TYR	513 TRP	516 ASP
Vasicinone	2	444 HIS	476 CYS	477 CYS	511 SER	513 TRP	516 ASP	
Vasicoline	3	388 ASP	391 TRP	444 HIS	477 CYS	511 SER	513 TRP	
Anisotine	3	388 ASP	390 SER	391 TRP	444 HIS	477 CYS	511 SER	513 TRP

Table 7. Docking Studies of Compounds Against FIMA – PDB 4Q98

Compounds	Binding Free Energy Kcal/mol	Inhibition Constant Ki µM (*mM)(**nM)	Electrostatic Energy Kcal/mol	Intermolecular Energy Kcal/mol	Total Interaction Surface
Vasicine	-5.37 kcal/mol	115.99 uM	-0.04 kcal/mol	-5.67 kcal/mol	521.66
Vasicinone	-6.69 kcal/mol	12.41 uM	-0.15 kcal/mol	-6.99 kcal/mol	519.072
Vasicoline	-7.54 kcal/mol	2.95 uM	-0.01 kcal/mol	-8.04 kcal/mol	664.194
Anisotine	-5.25 kcal/mol	141.27 uM	-0.05 kcal/mol	-5.38 kcal/mol	641.019

Table 8. Amino Acid Residue Interaction of Leads Against FIMA with PDB 4Q98

Compounds	Interaction	Amino Acid Residues										
Vasicine	1	237 TYR	317 PRO	319 LEU	325 ASN	363 PRO	364 GLN	367 GLN				
Vasicinone	1	237 TYR	317 PRO	319 LEU	325 ASN	327 TYR	363 PRO	364 GLN	367 GLN	368 ALA	370 LEU	
Vasicoline	1	237 TYR	317 PRO	319 LEU	325 ASN	327 TYR	363 PRO	364 GLN	367 GLN	368 ALA		
Anisotine	3	237 TYR	317 PRO	319 LEU	325 ASN	357 THR	360 PRO	363 PRO	364 GLN	367 GLN	368 ALA	370 LEU

Table 9. Summary of the Molecular Docking Studies of Compounds Against TNF-alpha (2AZ5)

Compounds	Binding Free Energy Kcal/mol	Inhibition Constant Ki µM (*mM)(**nM)	Electrostatic Energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Vasicine	-4.41 kcal/mol	581.81 uM	-0.19 kcal/mol	-4.71 kcal/mol	378.73
Vasicinone	-4.67 kcal/mol	376.54 uM	-0.07 kcal/mol	-4.97 kcal/mol	380.683
Vasicoline	-6.21 kcal/mol	27.83 uM	-0.04 kcal/mol	-5.89 kcal/mol	472.27
Anisotine	-6.25 kcal/mol	26.10 uM	-0.11 kcal/mol	-6.87 kcal/mol	606.919

Table 10. Amino Acid Residue Interaction of Lead Against TNF-alpha (2AZ5)

Compounds	Interaction	Amino Acid Residues					
Vasicine	3	59 TYR	61 GLN	119 TYR	151 TYR		
Vasicinone	4	57 LEU	59 TYR	61 GLN	119 TYR	120 LEU	151 TYR
Vasicoline	4	57 LEU	59 TYR	119 TYR	151 TYR	155 ILE	
Anisotine	4	57 LEU	59 TYR	61 GLN	119 TYR	151 TYR	

Discussion

With regard to computational analysis related to the use of AV in orthodontics, five targets were chosen. Primarily, the major complication is the dental caries as sequelae of orthodontic treatment. Caries are caused by multiple groups of microbes, of which *Streptococcus mutans* is the predominant pathogen. Glycosyltransferase (Gtf) is a critical virulence factor of *Streptococcus mutans*. Extracellular polysaccharides, especially glucans produced by *S. mutans* Gtfs, appear to contribute to the cariogenicity of dental biofilms, according to all available evidence. As a result, inhibiting Gtf activity and the resulting polysaccharide synthesis may reduce the virulence of cariogenic biofilms, which could be an alternative strategy for disease prevention caused by biofilms [17]. In this study binding of the four phytochemicals/leads with the said molecule was analysed.

It was observed that all 4 leads such as Vasicine, Vasicinone, Vasicoline and Anisotine reveal maximum of 3 interactions with the core active amino acid residues present on the target Glucosyltransferase (GFT) of *Streptococcus mutans*. Based on the results of the computational analysis it was concluded that the bio-active compound reveals significant binding against the target protein Glucosyltransferase (GFT) of *Streptococcus mutans*, thereby it was concluded that these compounds may exert promising to inhibit against GFT enzyme and hereby halt the catalytic transfer reactions which are essential for the survival of the organism *Streptococcus mutans*.

Periodontitis is caused by an inflammatory response to normal microbiota that is exacerbated by the presence of dysbiotic species. *P. gingivalis* is a "keystone pathogen" among these species, converting other benign biofilm members into pathobionts and causing aggressive damage to periodontal tissues.

Virulence factors of *P. gingivalis* include peptidases, which degrade proteins in infected tissues, nourishing bacteria and facilitating their spread and host colonisation. Peptidases also compromise host defences and outcompete bacterial competitors in periodontal pockets. The cysteine peptidases gingipain K are the most important (Kgp). Kgp is required for bacterial survival and the progression of periodontal disease, making it an ideal target for the development of new drugs [18].

Table 11. Summary of the Molecular Docking Studies of Compounds Against Prostaglandin H Synthases –PDB 1IGX

Compounds	Binding Free Energy Kcal/mol	Inhibition Constant Ki µM (*mM) (**nM)	Electrostatic Energy Kcal/mol	Intermolecular Energy Kcal/mol	Total Interaction Surface
Vasicine	-5.34 kcal/mol	121.37 uM	-0.50 kcal/mol	-5.64 kcal/mol	532.527
Vasicinone	-5.66 kcal/mol	70.64 uM	-0.10 kcal/mol	-5.96 kcal/mol	534.466
Vasicoline	-5.75 kcal/mol	60.71 uM	-0.03 kcal/mol	-5.37 kcal/mol	602.972
Anisotine	-6.51 kcal/mol	16.89 uM	-0.00 kcal/mol	-7.08 kcal/mol	630.438

Vasicoline and Anisotine revealed 100% binding efficacy by occupying the core amino acid residues (444 HIS, 477 CYS and 388 ASP) over the Gingipain K. Followed by compounds such as Vasicine and Vasicinone ranked second with the maximum of 2 interactions with the active site of the target enzyme Gingipain K. Vasicoline and Anisotine revealed significant binding affinity with all three active residual amino acids present on the active site of the target protein Gingipain K. Therefore, it was concluded that these compounds may exert promising inhibiting against Gingipain K.

The fimbriae are the main structures responsible for P. gingivalis's virulent behaviour. They adhere to epithelial cells, fibroblasts, salivary components, and collagen, and thus play an important role in periodontal tissue colonisation and invasion. The fim A gene encodes the main fimbria. Six genotypes of the FIM A gene have been identified based on the nucleotide sequence. Several studies have discovered links between periodontitis and a higher prevalence of fim A [19].

Nearly 24 amino acids are present in the sequence 339- 363 present in the active site of FIM A of *Porphyromonas gingivalis*. In our present investigation, it was observed that out of 4 compounds' the lead Anisotine reveals 3 potential interactions by occupying some of the active amino acid residues with the sequence (339- 363) present on the FIM A. Followed by this the compound Vasicine, Vasicinone and Vasicoline ranked second with the maximum of 1 interaction with the active site of the target protein FIM-A.

Anisotine revealed convincing binding affinity with the amino acids present on the active site of the target protein FIM-A and thereby it was concluded that the compound Anisotine may exert some productive efficacy in hindering the bio-film formation by the organism *Porphyromonas gingivalis*.

TNF- has been linked to the immunology of periodontal disease. TNF- concentrations may be elevated in periodontal inflammation as a side effect of monocyte stimulation. This cytokine's elevation affects insulin sensitivity through both direct and indirect mechanisms, worsening diabetic status. A worsening of the diabetic condition may result in further periodontal breakdown. Thus, TNF- appears to play a key role in the vicious cycle that connects periodontal disease and diabetes [20].

Vasicinone, Vasicoline and Anisotine possessed a maximum of four interactions followed by Vasicine which had three viable interactions with the core active amino acid residues present on the target TNF-alpha. Therefore, all the bio-active compounds revealed significant binding affinity against the target enzyme TNF-alpha by interacting with active amino acid present on the active site thereby it was concluded that these compounds may exert promising anti-inflammatory activity.

Table 12. Amino Acid Residue Interaction of Lead Against Prostaglandin H Synthases –PDB 1IGX

Compounds	Interaction	Amino acid Residues														
Vasicine	4	205 PHE	209 PHE	344 VAL	348 TYR	349 VAL	352 LEU	381 PHE	384 LEU	385 TYR	387 TRP	530 SER	534 LEU			
Vasicinone	4	205 PHE	344 VAL	348 TYR	349 VAL	352 LEU	381 PHE	385 TYR	530 SER	534 LEU						
Vasicoline	3	344 VAL	348 TYR	349 VAL	352 LEU	381 PHE	385 TYR	518 PHE	523 ILE	527 ALA	530 SER					
Anisotine	3	116 VAL	120 ARG	348 TYR	349 VAL	352 LEU	353 SER	355 TYR	381 PHE	384 LEU	385 TYR	387 TRP	523 ILE	527 ALA	530 SER	531 LEU
Salicylic acid	3	348 TYR	349 VAL	352 LEU	381 PHE	384 LEU	385 TYR	387 TRP	518 PHE	522 MET	530 SER					

PGE2 (prostaglandin E2) is a key inflammatory mediator in the pathophysiology of periodontitis. Three groups of enzymes act sequentially to catalyse PGE2 biosynthesis: phospholipase A2 (PLA2), cyclooxygenases (COX-1 and COX-2) and prostaglandin E (PGE) synthases, which act as catalysts in the final step of PGE2 synthesis. Therefore, inhibition of prostaglandin synthase H affects reducing periodontal inflammation [21].

Vasicoline, Anisotine, Vasicine and Vasicinone revealed a maximum of three to four interactions with the core active amino acid residues present on the target enzyme Prostaglandin H synthases. All the bio-active compounds revealed significant binding affinity against the target enzyme Prostaglandin H synthases by interacting with active amino acid present on the active site thereby it was concluded that these compounds may exert promising analgesic activity by inhibiting the activity of the enzyme Prostaglandin H synthases. It was concluded that the phytocomponents may act as potential therapeutic agents for the management of pain and inflammation.

From the above results it has been seen that Vasicine, Vasicinone, Vasicoline and Anisotine had a sufficient amount of Anti-Microbial activity towards cariogenic bacteria and periodontopathic bacteria. They also have analgesic and anti-inflammatory Activity. Therefore, they may find great use in the field of orthodontics.

Conclusion

The main point of the current research is that the compounds Vasicine, Vasicinone, Vasicoline, and Anisotine have significant binding affinity against various target enzymes related to cariogenic bacteria and periodontopathic bacteria, and they also possess analgesic and anti-inflammatory activity, making them potentially useful in the field of orthodontics. The said phytochemicals can further be explored for proper delivery to saliva, periodontal region and bone for effective use during and after orthodontic treatment.

Conflict of Interest

There is no conflict of interest.

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